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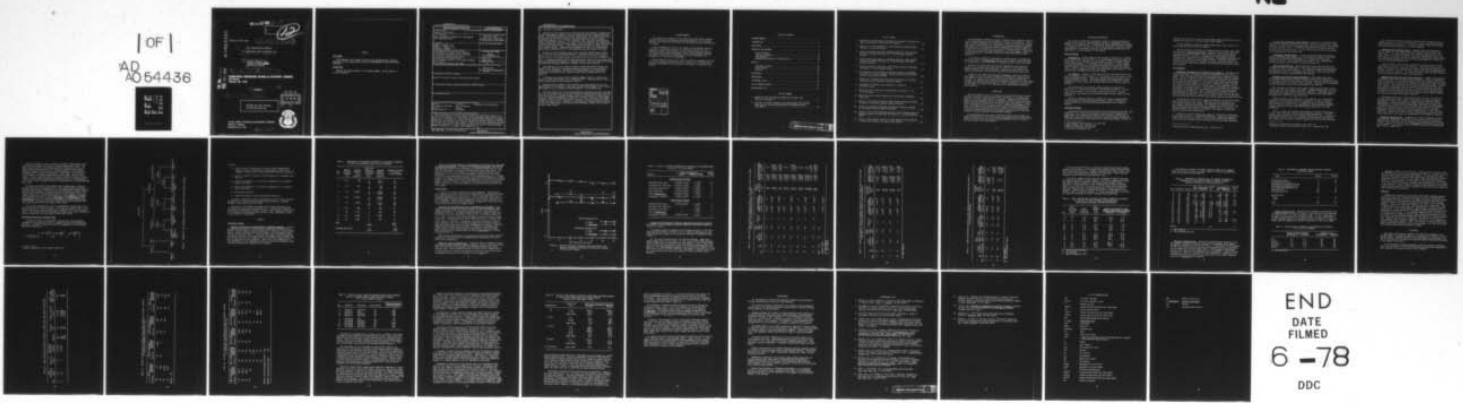

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TECHNICAL REPORT 7804

(14) USAMBRDL-TR-7804

(6) VIRAL AND BACTERIAL AEROSOLS AT A WASTEWATER SPRAY IRRIGATION SITE

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(9) Technical rept. Oct 75-Jul 76,

US ARMY MEDICAL BIOENGINEERING RESEARCH and DEVELOPMENT LABORATORY
Fort Detrick
Frederick, Md. 21701

(11) APRIL 1978

(12) 39p.

(16) 3E762720A835

(17) 00

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER TECHNICAL REPORT 7804	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) VIRAL AND BACTERIAL AEROSOLS AT A WASTEWATER SPRAY IRRIGATION SITE		5. TYPE OF REPORT & PERIOD COVERED Technical Report October 1975 - July 1976 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) HOWARD T. BAUSUM, Ph.D. STEPHEN A. SCHAUB, Ph.D. KATHERYN F. KENYON		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Medical Bioengineering Research & Development Laboratory, ATTN: SGRD-UBG Fort Detrick, Frederick, MD 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62720A 3E762720A835/00/136
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research & Development Command ATTN: AGRS-AJ Fort Detrick, Frederick, MD 21701		12. REPORT DATE APRIL 1978 13. NUMBER OF PAGES 37
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Aerosol	Coliform	Wastewater land application
Air sampling equipment	Spray irrigation	
Bacteria	Viruses	
Bacteriophage f2	Wastewater	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The second phase of a field study to measure microbiological aerosol dissemination by the spray irrigation mode of land wastewater application was conducted at Ft. Huachuca, AZ. Chlorinated and unchlorinated secondarily treated wastewaters were sprayed in periods of high and low sunlight intensity, and bacterial and virus aerosols were sampled. Predictions of long distance migration and aerosol dispersal of total aerobic bacteria and enteric virus (utilizing seeded coliphage f2 as a model), were emphasized.

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20. Abstract (continued)

Bacteria and coliphage aerosols were measured using Andersen stacked-sieve viable type impactor samplers and high volume electrostatic precipitator samplers. Preliminary laboratory studies indicated that the recovery efficiency of airborne f2 by Andersen samplers was 20-25 percent of that of liquid-impaction samplers, i.e., all-glass impingers (AGI) and high volume electrostatic precipitator samplers. In certain runs the wastewater was seeded with sodium fluorescein dye to estimate physical aerosol decay for later calculations of biological decay factors. This sampling was performed utilizing AGI and high volume samplers. Meteorological parameters as well as microbiological and chemical characteristics of the wastewater were monitored on each test.

Aerobic bacteria (standard plate count) averaged 2.4×10^5 colony-forming units (cfu)/ml in unchlorinated effluent. Bacterial aerosols were readily detected up to 150 m downwind from the sprayer and were as high as 9900/m^3 at 46 m. Coliphage averaged 4×10^5 plaque-forming units (pfu)/ml at the spray head and were detected 563 m downwind. Downwind aerosol levels in relation to source strength were somewhat enhanced by darkness or low solar radiation.

Chlorination, ca 6 mg/l total residual, reduced wastewater effluent bacterial levels 3300-fold, and reduced bacterial aerosols to near-background levels at all distances. Effluent f2 coliphage levels were reduced only 18-fold by chlorination and f2 aerosols were readily measured up to 137 m downwind.

Fluorescein dye aerosol levels remained somewhat higher in relation to source strength than bacterial or coliphage levels. They provided an indication of the extent of early bacterial die-off.

Median particle diameter in the aerosols was approximately 5.0 μm, and approximately 45-55 percent of the total bacteria and coliphage f2 fell within the range 1-5 μm, the range of maximal deposition in the human lung.

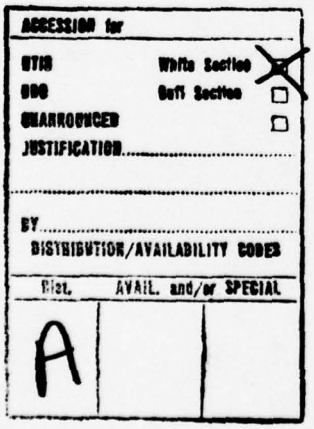
Utilizing those runs in which meteorological conditions were most satisfactory for the sampling period, source strength and meteorological data were used in conjunction with a dispersion model, based on the Pasquill diffusion equation, to generate mathematical predictions of aerosol strength at each sampler location. Ratios of predicted aerosol strength to observed aerosol strength ranged from 308 to 1661. Excesses of predicted over observed aerosols are due to the low aerosolization efficiency of the wastewater sprayer and biological aerosol decay factors.

ACKNOWLEDGMENTS

The authors are indebted to the following US Army Medical Bioengineering Research and Development Laboratory personnel: Mr. Kenneth Bartgis, for engineering assistance and SP4 Rudolph Spencer for technical assistance.

We are indebted also to Mr. E.W. Larson, US Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD and to Dr. C.A. Spendlove, Dugway Proving Ground, UT for helpful discussions and for providing certain sampling equipment items. Meteorological equipment and support rendered by the Atmospheric Sciences Laboratory, Ft. Huachuca, AZ is gratefully acknowledged.

Special thanks are due to Mr. Robert King, MAJ A. Amundsen and staff, Facilities Engineering Directorate, Ft. Huachuca, AZ for making many necessary arrangements and for providing assistance in support of the project.



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INTRODUCTION

Land wastewater treatment by spray irrigation is an important alternative in advanced wastewater treatment at military installations. From a public health standpoint, this technology raises the question of a potential human health hazard from inhalation of pathogen-containing aerosols. Some aspects of this problem have been addressed in previous studies[1-3] in which data were obtained on the travel of coliform bacteria from spray irrigation systems using undisinfected raw or settled sewage. There is a continuing need for field data affording a better definition of this problem.[4,5]

In an earlier study,[6,7] field data on aerosol levels of aerobic bacteria, coliforms, and Klebsiella during spray irrigation were presented. For comparison, a predictive model of downwind aerosol travel[8] was used.

The earlier field study[6] and the one reported here were both conducted at Ft. Huachuca, AZ, where the golf course is irrigated with ponded secondarily treated wastewater. Because of smooth terrain and other considerations, the driving range was selected as the test site.

In most of the tests reported here, wastewater leaving the holding pond pump pit was artificially seeded with coliphage f2, which was used as a model of aerosol migration and die-off for single-stranded RNA viruses. Air sampling was conducted at downwind distances up to 563 meters. In addition, data were obtained on the relative abundance of various bacterial groups in aerosols for comparison with corresponding wastewater concentrations.

OBJECTIVES

The first objective of this study was to provide field data on microbiological aerosols resulting from spray irrigation with treated wastewater at a military installation, to include: enumeration of bacteria present in the wastewater source; determination of the fraction of wastewater escaping the wetted zone as an aerosol; the measurement of aerosol levels and aerosol particle size of total, indicator and pathogenic bacteria; and comparison of wastewater and aerosol microbiological populations to provide information on relative rates of die-off through aerosolization, thereby contributing to eventual identification of the most suitable indicator organisms for wastewater aerosols.

A second objective was to use a bacterial virus tracer to model the aerosol dissemintation of enteroviruses by determining virus inactivation, aerosol particle size range, and estimated downwind travel of virus at relatively great distances downwind.

MATERIALS AND METHODS

This study included both laboratory studies at the US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) and field studies at Ft. Huachuca, AZ. Field studies were conducted from 19 October to 31 October 1975. Since this study is an extension of previous efforts conducted at Ft. Huachuca,[6,7] many of the experimental methods were the same and will not be repeated in this report.

General Methods

Coliphage f2. Bacteriophage f2 was prepared in quantity in tryptone-yeast extract (TYE) broth at 35°C in a batch fermentor using E. coli K13 as the host strain, according to the method of Loeb and Zinder[9] as modified by Enger et al.[10] For enumeration of coliphage in liquid samples, including those from the high volume electrostatic precipitator (LEAP)* sampler, routine soft agar plaque assay methods were used, with incubation at 35°C. E. coli strain K13, from logarithmic phase cultures in TYE broth at 35°C, served as the indicator system.

Air Samplers. Andersen viable type particle size-discriminating samplers,† operated at 28.3 liters of air per minute, were also used in estimating coliphage aerosols. These were loaded with plastic Petri dishes containing 45 ml TYE agar. Following exposure, and immediately before incubation at 35°C, plates were overlain with the soft agar-E. coli K13 mixture.

The electrostatic precipitator (LEAP) sampler was operated at 1000 l/min air flow and 13 kilovolts electrostatic potential. A 50 ml volume of one-quarter strength plate count broth‡ plus 0.1 percent Tween-80‡ was continuously recirculated through the LEAP sampler during operation.

All-glass impingers (AGI-30), drawing 12.5 l/min, contained 30 ml distilled water for sampling of dye aerosols or 30 ml of 50 percent nutrient broth plus one drop Dow-Corning Antifoam B for biological aerosols.

Laboratory Methods

Laboratory aerosols were generated by an all-glass two-fluid Pen-i-Sol$^{\delta}$ nebulizer or by a spinning disc aerosol generator (ERC)* operated at 60,000 RPM. All aerosols were dynamic with a constant airflow rate of 1000 l/min and passed through a 930 liter Plexiglas chamber fitted with transverse baffles near the entry ports to facilitate aerosol mixing. At

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* Environmental Research Corp., St. Paul, MN.
† Andersen 2000, Inc., Atlanta, GA.
‡ Difco Laboratories, Detroit, MI.
$^{\delta}$ Pen-i-Sol, Los Angeles, CA.

the effluent end of the chamber, an open-ended sampling manifold 10 cm in diameter was ducted to the high volume (LEAP) sampler.

In the laboratory, Andersen samplers operating at 28.3 l/min of air were located within the aerosol chamber.

The test bacterial aerosols were prepared from stationary phase shaking cultures of Serratia marcescens, pigmented strain, and E. coli 162, in plate count broth (Difco). Cells, freed of nutrient materials by 500-fold dilution, were held in secondarily-treated sewage (bio-disc effluent) for 1 hour prior to aerosolization. Coliphage f2 aerosols were prepared from seeded activated sludge effluent. Using the nebulizer, aerosols of median particle diameter 2.0 μm were generated, while, with the spinning disc generator, aerosols of median particle diameter 3.9 μm were prepared.

Field Methods

Wastewater Treatment and Irrigation at Ft. Huachuca. Wastewater was provided secondary treatment by trickling filtration and was stored in lagoons with a 6-8 day retention period, depending on irrigation requirements. During irrigation periods the wastewater flowed from the lagoons by gravity into a balance pond to aid in flow stabilization and then to the irrigation system pump pit. Centrifugal pumps forced the wastewater to the golf course through a 12-inch main. During this study, the flow was regulated to 550 gallons per minute (gpm) at 120 pounds per square inch (psi) at the pump station. Chlorination of wastewater was performed by addition of aqueous chlorine, formed by interaction of chlorine gas with water, at the pump pit. When it was desired to examine unchlorinated effluent at the spray site, the chlorine was shut off and the irrigation system operated for a period of at least 1 hour prior to sampling. This permitted the clearing of the distribution system of residual chlorine.

Five Rainbird* quick-set 85 E impact sprinklers with 1/2-inch nozzles were utilized during this study. Generally, only one sprinkler was utilized during each test period. The sprinkler was selected to best suit sampler location restrictions as a function of wind direction. The nozzle pressure was maintained at approximately 100 psi to provide an average "wetted" zone radius of 34 meters. The spray arc height was about 4.6 meters from ground level.

In runs in which air samples were drawn at a distance of 563 meters, five sprinklers were in simultaneous operation. These were arrayed in a single line, and on 46 m centers. Only the LEAP sampler was deployed at the furthest distance, with power obtained from a portable generator.

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* Rainbird Sprinkler Manufacturing Corp., Glendorra, CA.

f2 Virus and Fluorescein Tracer Studies. One end of a stainless steel tube with an internal diameter of 6 mm was fitted into the sprinkler system pump bell, and the other end extended above the pump pit where it was fitted to flexible rubber tubing. Continuous seeding of the sewage at the pump to obtain 10^5 pfu/ml f2 virus or 6 µg/ml fluorescein dye at the spray head was accomplished by peristaltic pump.

Fluorometric Determinations. In runs using added sodium fluorescein tracer, wastewater grab samples and LEAP and AGI air sampler fluids were subjected to fluorescence measurement in a fluorometer* fitted with exciter filters 2A + 47B and barrier filter 2A-12. The instrument was calibrated against dye solutions of known concentration.

Chlorine Determination. Determinations of total available and free available chlorine in wastewater at the spray nozzle were performed during evaluation of chlorinated aerosols. The LaMotte colorimetric DPD method[11] was employed in the field throughout each test run. Both total and free available chlorine were determined by this method.

Wastewater and Aerosol Sampling Methods. Grab samples were taken at the spray nozzle near the start and end of each aerosol sampling run. For samples of chlorinated effluent, sufficient sodium thiosulfate was added to the sample bottles to reduce 8 mg/l chlorine. Samples were refrigerated, and bacteriological analysis was performed on the same day.

For collection of bacteria from aerosol samples, both Andersen viable samplers and the LEAP samplers were used. Standard Methods agar† was used to determine the total aerobic bacterial population. Endo agar† was prepared daily for enumeration of coliforms. Tracer f2 were determined on TYE agar with a lawn of *E. coli* strain K13.

Andersen samplers were paired for field sampling, for simultaneous estimation of two different microbial parameters, at three distances downwind and at one upwind site. In addition, for each test, one downwind distance was chosen for the deployment of four additional Andersen samplers along an arc for either total aerobic bacteria or f2 virus estimation.

The high volume LEAP samplers were generally operated simultaneously at the closest and most distant downwind sampling stations. The LEAP samplers were operated at 13-14 kilovolts and 900-1000 l/min. Recirculating volumes of 50 or 100 ml of collection fluid were used, and evaporative losses were restored by frequent addition of sterile distilled water.

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* Model 110, Amsco/Turner Associates, Palo Alto, CA.
† Baltimore Biological Laboratories (BBL), Inc., Cockeysville, MD.

For fluorescein aerosols, Andersen samplers were replaced by AGI-30 samplers containing 30 ml distilled water. Excessive evaporative losses were restored during sampling. In the LEAP sampler, broth was replaced by distilled water containing 0.1 percent Tween-80.

Sampler Stands and Power Supply. Sampler stands were prepared so the air intakes of AGI, Andersen and LEAP samplers were 5.5 feet from ground level. The stands were made to be readily broken down for shipping. While all stands and samplers were kept approximately level, the LEAP sampler stands were fitted with additional adjustable leveling screws to allow precise adjustments because of the requirement for a level surface for the wetted sampling disc.

Electrical power (115V AC) was supplied to the test site by an electrical drop from a power line to a fuse box. Three-wire cable carried power from the box to a junction where leads to the downwind samplers were joined. A smaller cable from the golf course club house provided power to the upwind control samplers. All downwind samplers were started simultaneously by throwing the main switch on the fuse box.

Meteorological Monitoring and Test Conditions. Sampling was performed at different periods of the day so that various air stability conditions could be observed. On-site meteorological recording systems were activated several hours prior to sampling to determine if appropriate sampling conditions existed. Most important was the wind direction measurement. Generally, sampling procedures were initiated only when there was less than 90 degrees of directional variability. If this criterion was satisfied, the samplers were positioned at the mean position of the wind direction. At night, the driving range lights were turned on during sampler preparation and extinguished during actual sampling.

Continuous general area meteorological data and local site data were collected during the study. Local field devices provided continuously recorded wind speed, wind direction, temperature and relative humidity measurements. Wind measurements were taken 2 meters from ground level. Temperature and relative humidity measurement were taken at approximately 1.2 meters from ground level, using devices contained within a shelter. General area meteorological data were collected at the Ft. Huachuca Atmospheric Sciences Laboratory, located approximately 3 miles from the spray site. General area data collected included total and direct radiation, evaporation rate, upper air measurements, and barometric pressure.

Bacterial Identification. Enumeration of total presumptive coliform organisms was performed on secondary effluent samples using the spread plate method on Endo agar and the membrane filter method on m-Endo broth (Difco). Fecal coliforms and fecal Streptococcus determinations were made by the membrane filter method using m-FC broth (Difco) and m-Enterococcus agar (Difco), respectively.

Bacteria isolated in pure cultures for partial identification were obtained from colonies on plates of Standard Methods agar (BBL). For each such plate used, all colonies present on the plate, or on a randomly chosen sector thereof, after 48 hours incubation were picked, thereby eliminating bias based on colony morphology. In the case of Andersen samples, colonies were chosen in this manner from each plate in the sample, in numbers proportional to that plate's share of the sample. In this way, bias based on aerosol particle size was minimized. Prior to testing, each colony was purified by isolation streaking and cells from a single isolated colony were transferred to a Standard Methods agar slant.

Isolates were classified according to the schema shown in Figure 1, which is based on key characteristics. The scheme begins by dividing Gram-positive bacteria according to cellular morphology and Gram-negative bacteria into fermenters, oxidizers and non-utilizers of glucose. Furthere subdivision of Gram-negative bacteria relied largely on the cytochrome oxidase test, which distinguishes not only Aeromonas and Vibrio from Enterobacteriaceae, but most Alkaligenes and Flavobacterium from Acinetobacter, etc. The categories defined do not always follow genus lines, and no isolate could be fully identified without additional testing, which was not within the scope of this study.

The oxidative/fermentative test was performed in paired tubes of O-F agar (Difco) with 1 percent glucose. One tube was overlain with 1 cm of sterile mineral oil, and the tubes were incubated at 35°C for 24 and 48 hours. The oxidase test was performed using paper test strips (Pathotec*), and the citrate test, using Simmons citrate agar (Difco).

Mathematical Analysis of Aerosol Data

A dispersion model was developed[8] to account for the performance parameters of the sprayer, as well as sampler location and meteorological data. The model is based on Turner's[12] adaptation of the diffusion equation of Pasquill:[13]

$$C = \frac{Q}{2\pi\,\sigma_y\,\sigma_z\,U}\left[e^{-0.5\left(\frac{y}{\sigma_y}\right)^2}\right]\left[e^{-0.5\left(\frac{Z-H}{\sigma_z}\right)^2} + e^{-0.5\left(\frac{Z+H}{\sigma_z}\right)^2}\right]$$

* General Diagnostics, Inc., Morris Plains, NJ.

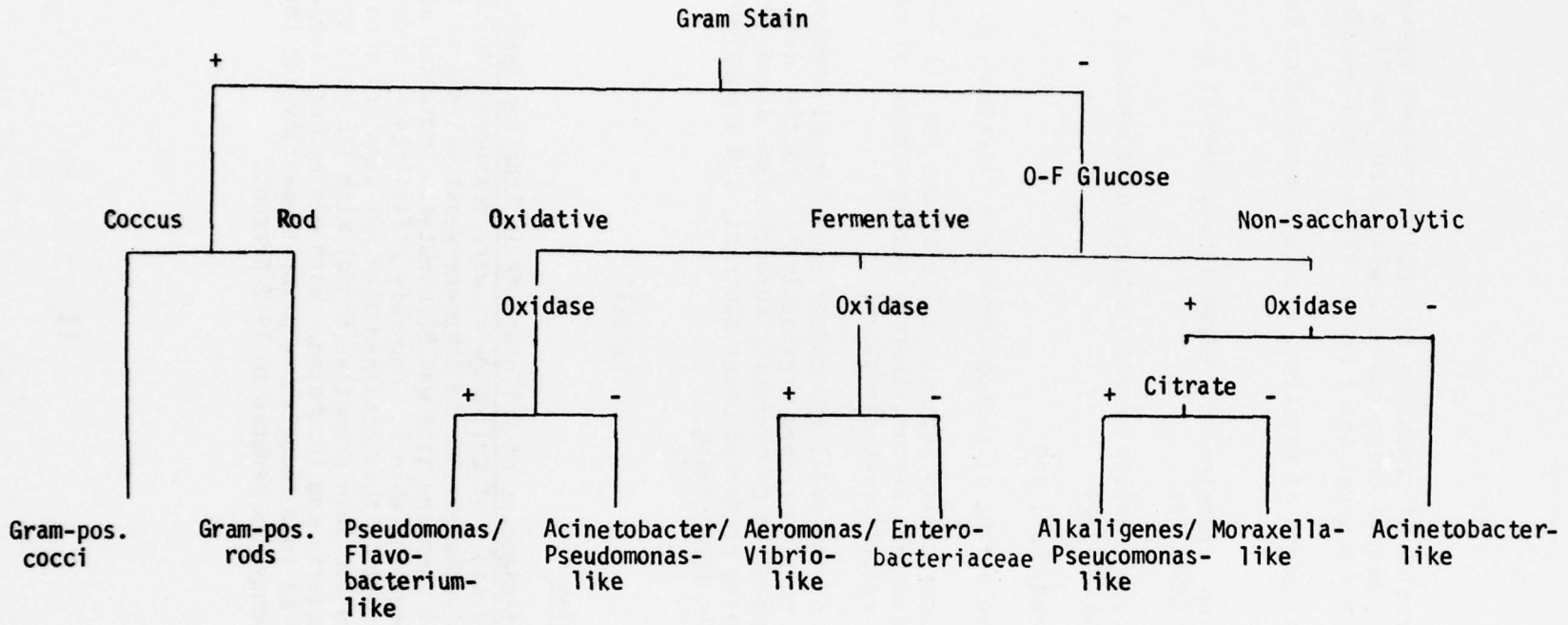

Figure 1. Schema for the Assignment of Bacterial Isolates into Arbitrary Groupings.

in which:

C = concentration of organisms at a point x meters downwind of a source, y meters crosswind of a wind-direction line running through the source, and z meters above zero elevation, counts/m^3;

Q = source strength of emission, viable counts/sec or nanograms (ng) dye/sec;

σ_y = dispersion parameter in y direction, dependent on x and atmospheric stability;

σ_z = dispersion parameter in z direction, dependent on x and atmospheric stability;

U = wind speed, mps; and

H = effective height of source above zero elevation, m.

The model incorporates separate calculations for all 1-min intervals throughout the sampling period, thereby taking account of recorded changes in wind velocity and direction.

Application of the model and comparison of model predictions with field data have been described previously.[6,7] Further data evaluation and predictive modeling of microbial aerosol plume dispersion and die-off are currently being performed under contract, and a supplemental report on this effort is forthcoming.

RESULTS

Laboratory Studies

Recovery Efficiencies of Coliphage f2 in Andersen Samplers. A series of laboratory aerosols of coliphage f2 were measured simultaneously by Andersen and AGI samplers, using tryptone-yeast extract as the collecting medium. Aerosol sampling time was 30 minutes. Indicated aerosol densities are presented in Table 1. Recovery efficiency in Andersen samplers averaged 28 percent of that obtained with AGI samplers when Andersen sampler plates were overlain promptly (5 min) with TYE soft agar containing host E. coli bacteria and incubated. When plates were subjected to a 45 minute delay at room temperature prior to overlay and incubation, mean recovery efficiency was reduced to 19.2 percent.

TABLE 1. COMPARISON OF SIMULTANEOUS ESTIMATES OF COLIPHAGE f2 AEROSOLS BY AGI-30 AND ANDERSEN VIABLE-TYPE AIR SAMPLERS

Exp. No.	Median Particle Diameter (μm)	Aerosol Density, AGI (pfu/m^3)	Time from Exposure to Overlay, Andersen (min)	Aerosol Density, Andersen (pfu/m^3)	Andersen Estimate as Percent of AGI Estimate
1	3.9	89	5	22.4	25
			45	16.5	19
2	2.0	710	5	128	18
			45	86	12
3	2.0	14,450	5	2,860	20
			45	3,180	22
4	3.9	14,400	5	4,900	34
			60	2,700	19
5	3.9	3,400	5	1,310	38
			60	816	24
6	3.9	388	5	106	27
7	3.9	1,712	5	480	28
8	3.9	6,000	5	1,988	33
9	3.9	1,520	5	475	31
Mean			5		28.2
			45-60		19.2
Standard Deviation			5		6.55
			45-60		4.55

Effect of Prolonged Exposure in LEAP Sampler on Survival of E. coli 162 and Serratia marcescens. E. coli 162 and Serratia marcescens from 16-hour shaking broth cultures were continuously circulated through the LEAP sampler for 30 minutes in one-fourth strength Difco plate count broth. One thousand liters per min airflow and 13 KV electrostatic potential were maintained. In the first of two experiments, inoculation of the circulating fluid was made directly and, in the second experiment, by a 2-minute exposure to a dynamic aerosol generated from sterilized secondarily treated sewage. All samples were held at room temperature for the entire 30 minutes. Results, shown in Figure 2, indicate no decline in bacterial recovery by either very short or long-term exposure to conditions existing within the LEAP sampler.

Field Studies

A total of 15 aerosol sampling runs were completed. Total bacteria were enumerated during all runs, while seeding with the coliphage f2 tracer was conducted during 11 of the 15 runs. Nine of the sampling runs were conducted under daylight conditions (1.3 to 2.4 Langleys). Four runs, all with coliphage f2 present, were conducted at dusk or under darkness (0.5 to 0.7 Langleys). Chlorinated wastewater was examined in three runs only, of which two included the coliphage tracer.

Bacterial and Coliphage Densities in Wastewater Grab Samples. Mean levels of various groups of bacteria present in unchlorinated wastewater samples obtained at the spray nozzle during aerosol determinations are presented in Table 2. Coliphage determinations on E. coli K13 were performed only for those runs where wastewater was artificially seeded with f2. A mean level of 4.1×10^5 pfu/ml wastewater was achieved during these seeded runs. Standard bacterial plate count varied over a tenfold range with a mean of 2.4×10^5/ml. Presumptive coliforms enumerated on M-Endo broth represent a more valid coliform estimate than do those counted on Endo agar, which were a more inclusive assemblage of organisms. Mean presumptive coliform densities were about 1.2 percent of standard plate counts, while, of the former, about 8.2 percent were fecal coliforms. Fecal coliform levels exceeded those of fecal Streptococcus by a factor of approximately 18.

When chlorinated effluent was examined, mean reductions below unchlorinated levels were 3300-fold for standard plate count bacteria but only 18-fold for coliphage f2.

Bacterial Levels in Ambient Air. Bacterial levels in ambient air were determined by positioning two Andersen samplers upwind of the spray source. These were operated simultaneously with downwind samplers during all sampling runs. These background bacterial aerosol levels ranged from 15 to 198 cfu/m^3 for coliform-like organisms on Endo agar and also for bacteriophage infective for the indicator strain E. coli K13.

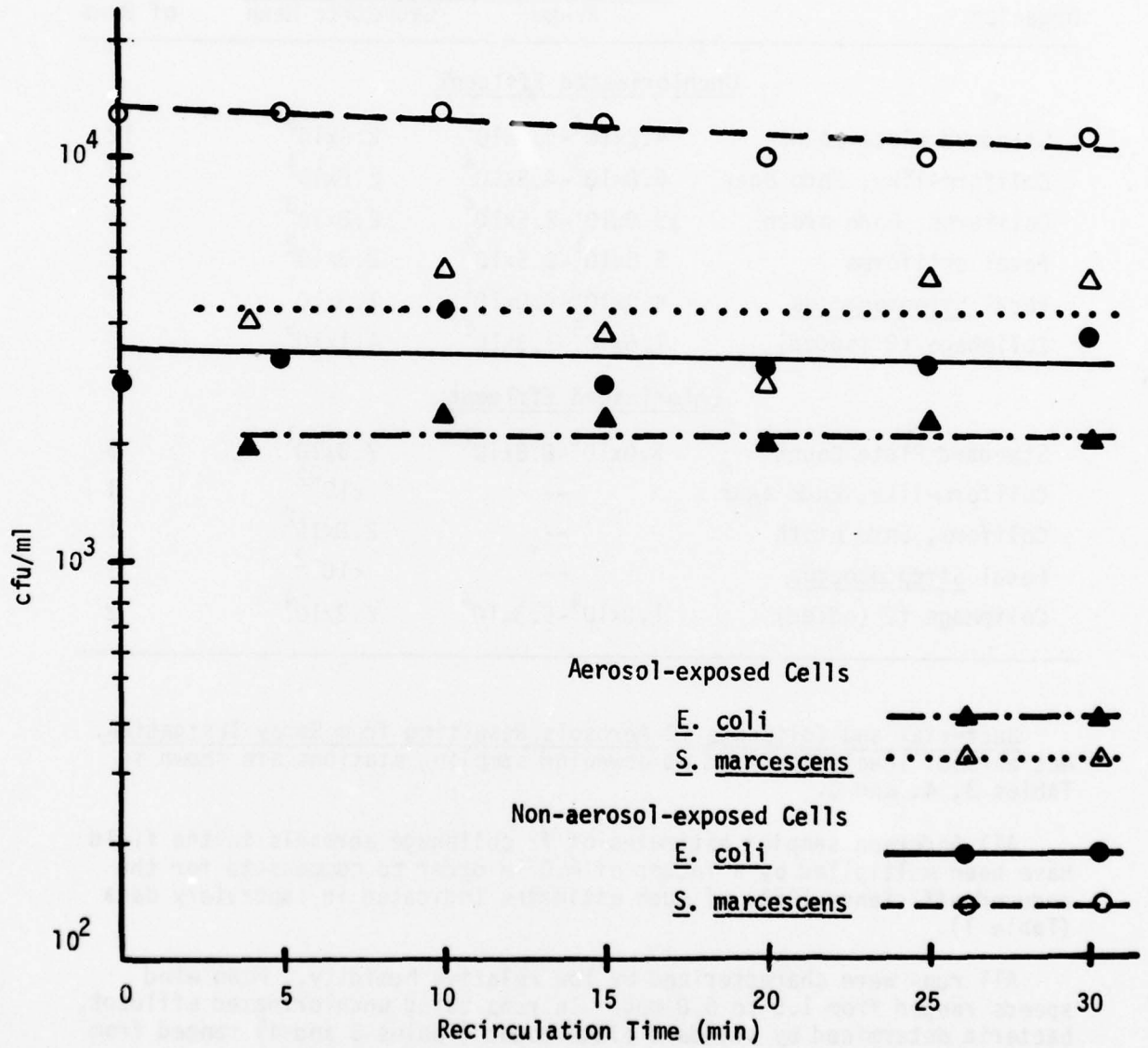

Figure 2. Survival of Enteric Bacteria, with and without Prior Aerosol Challenge, on Continual Recirculation Through Operating LEAP Sampler.

TABLE 2. DENSITY OF VARIOUS ORGANISMS IN CHLORINATED AND UNCHLORINATED EFFLUENT AT THE SPRAY NOZZLE

Organism	Viable Organisms/ml		Number of Runs
	Range	Geometric Mean	
Unchlorinated Effluent			
Standard plate count	4.2×10^{4}-4.1×10^{5}	2.4×10^{5}	12
Coliform-like, Endo agar	6.8×10^{3}-4.8×10^{4}	2.2×10^{4}	3
Coliforms, Endo broth	9.0×10^{1}-2.5×10^{4}	2.8×10^{3}	3
Fecal coliforms	5.5×10^{1}-9.5×10^{2}	2.3×10^{2}	2
Fecal Streptococcus	4.0×10^{0}-4.0×10^{1}	1.3×10^{1}	3
Coliphage f2 (added)	1.6×10^{5}-1.3×10^{6}	4.1×10^{5}	9
Chlorinated Effluent			
Standard Plate Count	5.0×10^{1}-8.8×10^{1}	7.3×10^{1}	3
Coliform-like, Endo agar	--	$<10^{-2}$	1
Coliform, Endo broth	--	2.0×10^{2}	1
Fecal Streptococcus	--	$<10^{-2}$	1
Coliphage f2 (added)	1.0×10^{4}-5.3×10^{4}	2.3×10^{4}	2

Bacterial and Coliphage f2 Aerosols Resulting from Spray Irrigation. Net aerosol levels observed at downwind sampling stations are shown in Tables 3, 4, and 5.

All Andersen sampler estimates of f2 coliphage aerosols in the field have been multiplied by a factor of 4.0 in order to compensate for the reduced efficiency (25%) of such estimates indicated in laboratory data (Table 1).

All runs were characterized by low relative humidity. Mean wind speeds ranged from 1.5 to 5.0 mps. In runs using unchlorinated effluent, bacteria determined by standard plate count (Tables 3 and 4) ranged from 150 to 10,500 colony-forming particles (cfp)/m^3 above background at 46 m downwind. The same parameter reached up to 4,700 at 76 m, 3,200 at 101 m, 500 at 152 m, and 13 at 563 m downwind. Coliphage f2 recovery was as high as 19,000 plaque-forming particles (pfp)/m^3 at 46 m and 460 at 563 m downwind.

TABLE 3. SOURCE AND AEROSOL DENSITIES (ANDERSEN SAMPLER), TOTAL AEROBIC BACTERIA AND ADDED COLIPHAGE f2 IN UNCHLORINATED EFFLUENT, DAYTIME

Run	RH (%)	Total Radiation (Langleys)	Mean Wind Speed (mps)	Stability Class	Source Strength		Sampler Distance from Source (m)	Net Aerosol Strength	
					Bacteria (cfu/ml)	Coliphage (pfu/ml)		Bacteria (cfp/m^3)	Coliphage (pfp/m^3)
2	19	1.9	4.0	C	4.0×10^5	--[a]	46	4.4×10^3	--[a]
							92	1.8×10^1	--[a]
4	27	1.6	4.3	C	2.7×10^4	4.4×10^5	46	1.5×10^2	9.6×10^2
							76	1.2×10^2	3.9×10^2
							152	1.0×10^0	4.0×10^1
11	13	1.4	1.5	B	3.6×10^5	1.8×10^5	46	2.6×10^3	8.8×10^3
							76	8.8×10^2	6.0×10^3
12	26	2.4	3.1	B	1.7×10^5	--[a]	46	2.2×10^2	--[a]
							76	9.7×10^2	--[a]
							101	1.8×10^2	--[a]
13	13	2.3	5.0	BC	4.0×10^5	1.6×10^5	46	1.2×10^3	1.2×10^3
							76	4.7×10^3	1.1×10^3
							101	1.9×10^3	1.4×10^3
14	20	1.2	3.5	C	1.6×10^5	--[a]	46	1.0×10^4	--[a]
							76	3.8×10^3	--[a]
							101	3.2×10^3	--[a]
16	15	2.2	4.6	B	4.2×10^4	2.3×10^5	46	1.5×10^2	--[a]
							76	3.3×10^1	--[a]
							99	1.1×10^1	--[a]
							563	1.0×10^1	5.3×10^0[b]
17	13	1.3	5.0	CD	1.1×10^5	2.5×10^5	46	7.5×10^2	8.8×10^2
							563	1.3×10^1[b]	4.6×10^2[b]
Mean	18.3	1.8	3.9						
Geometric Mean					1.24×10^5	2.28×10^5			

a. Not sampled.
b. LEAP sampler.

TABLE 4. SOURCE AND AEROSOL DENSITIES (ANDERSEN SAMPLER), TOTAL AEROBIC BACTERIA AND ADDED COLIPHAGE f2, IN UNCHLORINATED EFFLUENT, DUSK AND DARKNESS

Run	RH (%)	Total Radiation (Langleys)	Mean Wind Speed (mps)	Stability Class	Source Strength		Distance from Spray Source (m)	Aerosol Strength	
					Bacteria (cfu/ml)	Coliphage (pfu/ml)		Bacteria (cfp/m^3)	Coliphage (pfp/m^3)
3	20	0.7	3.8	C	4.0×10^5	4.9×10^5	46	4.2×10^3	1.9×10^4
							76	--[a]	9.6×10^3
5	32	0.7	2.8	C	2.4×10^5	6.7×10^5	46	3.8×10^3	1.1×10^3
							76	2.9×10^2	8.6×10^2
							152	5.0×10^2	8.4×10^1
9	5	0.5	1.9	BC	3.6×10^5	9.9×10^5	46	1.1×10^4	1.2×10^4
							76	3.9×10^3	3.3×10^3
							152	2.6×10^2	0
10	6	0.5	2.1	C	4.1×10^5	1.3×10^6	46	9.9×10^3	7.5×10^3
							61	1.9×10^3	5.6×10^3

a. Not sampled.

TABLE 5. SOURCE AND AEROSOL DENSITIES, TOTAL AEROBIC BACTERIA AND ADDED COLIPHAGE f2 IN CHLORINATED EFFLUENT

Run	RH (%)	Total Radiation (Langleys)	Mean Wind Speed (mps)	Stability Class	Chlorine Residual (mg/l) Total	Chlorine Residual (mg/l) Free	Source Strength Bacteria (cfu/ml)	Source Strength Coliphage (pfu/ml)	Distance from Spray Source (m)	Aerosol Strength Bacteria (cfp/m^3)	Aerosol Strength Coliphage (pfp/m^3)
6	24	2.4	2.1	A	6.0	0.7	8.8×10^1	--[a]	46	5.7×10^1	--[a]
										2.8×10^1	--[a]
7	18	1.7	1.5	B	6.5	0.8	8.7×10^1	1.0×10^4	46	3.1×10^1	1.6×10^3
									137		9.6×10^1
15	22	2.3	4.6	B	6.0	0.6	5.0×10^1	5.3×10^4	46	--[b]	5.1×10^2
									76	--[b]	4.4×10^2
									101	--[b]	2.0×10^2

a. Not sampled.
b. Below background levels.

From chlorinated effluent, coliphage f2 aerosols were readily demonstrated at distances up to 101 meters, the greatest distance tested under those conditions (Table 5). Standard bacterial plate count results, however, fell to near-background levels at all distances. Total chlorine residual at the nozzle was approximately 6 mg/l.

In Table 6, standard plate count and coliphage f2 aerosol densities have been normalized to source strength. There was a tendency for Andersen sampler standard bacterial plate counts to be somewhat higher in relation to source strength in runs characterized by dusk or darkness (Runs 3, 5, 9, and 10). In runs 6, 7, and 15, using chlorinated wastewater, bacterial aerosols were extremely high in relation to source strength. However, due to inherent variability in measurements of the ambient level of airborne bacteria, it is not clear how much of the aerosol observed represents an actual increase above background.

TABLE 6. TOTAL BACTERIA AND COLIPHAGE f2 AEROSOL STRENGTH AT 46 METERS, IN RELATION TO SOURCE STRENGTH, CHLORINATED AND UNCHLORINATED EFFLUENT, DAYTIME AND EVENING

Run	Total Chlorine Residual (mg/l)	Total Radiation (Langleys)	Mean Aerosol Time (sec)	Viable Particles/m^3 X 1000 / Viable Particles/ml at Source	
				Bacteria	Coliphage
2	0[a]	1.9	11.5	11.0	--[b]
4	0	1.6	10.7	7.9	2.6
11	0	1.4	30.7	7.2	49.0
13	0	2.3	9.2	7.5	7.5
14	0	1.2	13.1	6.3	--[b]
16	0	2.2	10.0	3.6	--[b]
17	0	1.3	9.2	6.8	3.5
3	0	0.7	12.1	10.5	39.0
5	0	0.7	16.4	15.8	6.4
9	0	0.5	24.2	30.1	11.9
10	0	0.5	21.9	24.1	5.8
6	6.0	2.4	21.9	647.0	--[b]
7	6.5	1.7	30.7	356.0	150.0
15	6.0	2.3	10.0	--[c]	9.6

a. Unchlorinated effluent.
b. Not sampled.
c. Below background level.

LEAP sampler estimates of aerosol strength tended to be somewhat lower than Andersen sampler estimates (Table 7) for both standard bacterial plate count and coliphage f2.

TABLE 7. COMPARISON OF ANDERSEN AND LEAP SAMPLER ESTIMATES OF STANDARD PLATE COUNT AND COLIPHAGE f2 AEROSOL STRENGTH. ANDERSEN ESTIMATES OF f2 MULTIPLIED BY 4.0

Run	Distance	Azimuth	Std. Plate Count cfu/m^3			Coliphage f2		pfu/m^3
			LEAP	Andersen	A/L	LEAP	Andersen	A/L
4	76	68	--[b]	124	∞	343	392	1.1
5	76	70	1,400	2,880	2.1	2,280	456	0.2
6	46	291	--[b]	57	∞	--[a]	--[a]	
7	46	253	--[b]	--[b]		32	312	9.8
9	76	93	4,070	3,930	0.97	11,700	3,316	0.3
10	50	113	2,100	1,870	0.89	20,000	5,520	0.3
11	76	328	344	3,370	9.8	3,046	6,000	2.0
12	76	313	53	502	9.5	--[a]	--[a]	
12	101	313	108	183	1.7	--[a]	--[a]	
13	76	313	702	4,730	6.7	295	1,072	3.6
13	101	313	35	1,900	54.3	0	1,372	∞
14	76	313	778	3,800	4.9	--[a]	--[a]	
14	101	313	388	3,210	8.3	--[a]	--[a]	
15	76	313	--[b]	--[b]		30	444	14.8
15	101	313	--[b]	--[b]		173	200	1.2
16	76	315	--[b]	--[b]				
Median					7.5			1.6

a. Not sampled.
b. Below background level.

Bacterial Identification. Results of bacteriological testing of random samples of bacterial colonies obtained from wastewater grab samples and aerosol samples are presented in Table 8. A significant shift occurred in the overall distribution of bacteria into groups ($P<0.01$, as indicated by chi-square based on contingency table). This suggests differential die-off rates among various components of the population. Major relative increases, in aerosol as compared to source populations, occurred for the Enterobacteriaceae group and Gram-positive rods, while a major decrease occurred for Moraxella-like bacteria.

TABLE 8. ASSIGNMENT OF RANDOMLY CHOSEN BACTERIAL ISOLATES TO ARBITRARY GROUPS

Group	Source	Aerosol
Enterobacteriaceae	2	34
Aeromonas/Vibrio-like	5	17
Pseudomonas/Flavobacterium-like	0	1
Acinetobacter/Pseudomonas-like	0	3
Alkaligenes/Pseudomonas-like	1	3
Moraxella-like	54	11
Acinetobacter-like	35	46
Gram Positive		
Rods	2	14
Cocci	7	4
Total	106	132

Aerosol Particle Size. Median particle diameters for bacterial and coliphage aerosols are given in Table 9. Also included are percentages of particles falling in the respirable range, 1-5 μm.[14] Each value is the mean of observations for a number of runs. No great difference is evident between experimental and upwind bacterial aerosols in regard to either particle diameter or percent of particles in the 1-5 μm range.

TABLE 9. MEDIAN PARTICLE DIAMETER AND PERCENTAGE OF PARTICLES IN 1.0 TO 5.0 μm RANGE

	Median Particle Diameter		Percent 1-5 μm	
	Coliphage f2	Bacteria	Coliphage f2	Bacteria
46 m	4.7	5.1	56	48
61-76 m	5.1	5.3	45	44
92-101 m	4.1	5.5	62	43
143-152 m	5.9	4.8	39	50
Upwind	--[a]	5.1	--[a]	42

a. Not determined.

Dye Runs. Results of the two runs in which aerosols of tracer fluorescein dye were generated are summarized in Table 10. Aerosol levels at 46 m are mean values of several measurements. In relation to source strength, fluorescein aerosol levels were higher than bacterial and coliphage aerosols (cf. final column of Table 10 and final two columns of Table 6). The difference is a reflection of the existence of a biological die-off factor in microbial aerosols and the absence of such a factor in dye aerosols. Direct comparison cannot be made, however, as microbiological and dye aerosols were not measured in the same runs nor under the same meteorological conditions.

Modeling

Mathematical modeling of runs having sufficiently complete meteorological and other data yielded predictions of aerosol concentration at each sampler location. For each such sample, the ratio predicted concentration/observed concentration was derived, and these ratios are presented in Table 11 for bacterial aerosols, and Table 12 for coliphage aerosols. Ratios for individual samplers vary over a wide range. This is particularly true within runs (cf. runs #2 and 3 in Table 11 and #10 in Table 12). Thus, the mathematical apportionment of airborne microorganisms to various regions of the sampling array differed greatly from the actual distribution. If all downwind samples taken in a given run are treated as one composite sample, and a composite prediction is used, an overall ratio predicted recovery/observed recovery is derived for each run. Such ratios are given in Table 13. The ratios for the bacterial runs (median = 626) are quite comparable to those for the coliphage runs without chlorination (median = 639). The ratio of coliphage from the run using chlorinated effluent, run #7, was much lower, i.e., 79, which indicates greater than predicted aerosol survival.

DISCUSSION

Only about 20-25 percent recovery of aerosolized coliphage f2 was accomplished by the Andersen samplers in comparison to recovery in liquid AGI-30 samples. Andersen sampler collection efficiency for bacteria, as well as collection efficiencies for the LEAP sampler, approach 100 percent of AGI sampler efficiency.[6]

In the secondarily treated wastewater, both the bacterial concentrations and the effect of chlorination upon the bacteria were comparable to those reported in the earlier phase of this study.[6,7]

TABLE 10. MEAN FLUORESCEIN AEROSOL RECOVERIES, SOURCE DATA AND AMBIENT CONDITIONS

Run	Mean Wind Speed (mps)	Stability Class	Dye Level at Nozzle (ng/ml)	Sampler	Distance (m)	Aerosol Strength (ng/m^3)	ng/m^3 X 1000 ng/ml effluent
1	2.2	A	9.4×10^2	AGI	46	3.1×10^2	333
8	2.6	C	3.9×10^3	AGI	46	3.6×10^2	92
				AGI	76	1.4×10^2	36
				LEAP	46	9.7×10^1	25

TABLE 11. RATIOS OF PREDICTED TO OBSERVED TOTAL AEROBIC BACTERIAL AEROSOL STRENGTH AT INDIVIDUAL ANDERSEN SAMPLER STATIONS

Run 2		Run 3 (Low Sun)		Run 9 (Darkness)		Run 12	
Distance	Predicted / Observed	Distance	Predicted / Observed	Distance	Predicted / Observed	Distance	Predicted / Observed
46	24	46	1,060	46	360	101	1,160
46	354	52	6	152	36	76	720
46	2,320			76	188	46	3,870
46	9,750					76	180
Median: 357							

TABLE 12. RATIOS OF PREDICTED TO OBSERVED COLIPHAGE f2 AEROSOL STRENGTH AT INDIVIDUAL ANDERSEN SAMPLER STATIONS

Run 3 (Low Sun)		Run 7 (Chlorinated)		Run 9 (Darkness)		Run 10 (Darkness)		Run 13	
Distance	Predicted / Observed	Distance	Predicted / Observed	Distance	Predicted / Observed	Distance	Predicted / Observed	Distance	Predicted / Observed
76	580.0	46	54	46	1,860	46	383.0	101	309
46	432.0	46	141	46	1,885	46	503.0	76	536
46	305.0	46	147	152	448	46	223.0	46	763
52	2.8	46	120	76	581	46	0.8	76	675
		76	1,046			61	0		
		46	32			46	2564.0		
		137	42			46	1095.0		
Median, non-chlorinated effluent:			536						
Median, chlorinated effluent:			120						

TABLE 13. RATIOS OF TOTAL PREDICTED RECOVERY/TOTAL ACTUAL RECOVERY WHEN ALL DOWNWIND SAMPLES FROM EACH RUN ARE POOLED AS A SINGLE COMPOSITE SAMPLE

Run	Organism	Radiation	Chlorination	Predicted Recovery / Actual Recovery
2	Bacteria	Daylight	No	669
3	Bacteria	Dusk	No	1,047
9	Bacteria	Darkness	No	308
12	Bacteria	Daylight	No	582
3	Coliphage	Dusk	No	364
7	Coliphage	Daylight	Yes	79
9	Coliphage	Darkness	No	1,661
10	Coliphage	Darkness	No	734
13	Coliphage	Daylight	No	544

The value of coliphage f2 as a tracer virus is evident in this work. Coliphage aerosols downwind are demonstrable at lower levels than total bacterial aerosols because of the virtual absence of a background coliphage. Thus, in this study, samples taken at the long distance (563 m) stations yielded an obvious contribution above background for f2 phage but a questionable contribution from wastewater-borne bacteria.

The resistance of this coliphage to chlorination (Table 2) was observed to be much greater than that of the standard aerobic bacterial plate count or the indicator bacteria tested. This factor, coupled with relatively good aerosol survival, makes f2 an effective label for chlorinated wastewater aerosol evaluation. High resistance to chlorination is a property of some pathogenic microorganisms, including enteric viruses, acid-fast bacteria, bacterial spores, and amebic cysts. A marker with similarly high chlorine resistance is a better model than the coliform group for the determination of downwind migration of such resistant pathogens from chlorinated wastewater.

Bacterial aerosol levels downwind were generally comparable to those observed in the first phase of the study. Also, as in the earlier phase, bacterial aerosols in relation to source strength were higher under dusk or night conditions (Table 6) than during the day. Low relative humidity prevailed during both daytime and night runs (Tables 4, 5, and 6). Night runs were, however, characterized by low radiation, lower wind speeds and greater wind stability. The relative contributions of these various factors to the enhanced aerosol levels observed at night cannot be accurately resolved at this time.

Bacterial aerosols observed in the three runs using chlorinated effluent (6.0-6.5 mg/l total chlorine) do not represent significant increases above background. Nonzero net aerosol levels of 28 to 57 cfu/m^3 were recorded at 46 m (Table 5), but background levels of 40-57 cfu/m^3 in the same runs, coupled with high sampler-to-sampler variability, render the result uncertain. The same uncertainty exists with respect to bacterial aerosols, from unchlorinated effluent, at the 563 m distance (Table 4). Corresponding seeded coliphage aerosols, however, were far above background levels in both cases.

One objective of these studies concerns the identification of groups of microorganisms suitable for use as indicators of microbial aerosols arising from wastewater sources. Several criteria have been recognized as important in such a group, e.g., prevalence in sewage, relatively good resistance to aerosolization and chlorination stresses, and scarcity in the ambient microbial aerosol. Ultimately, an indicator system must reflect a persistent, identifiable addition to the ambient aerosol.

The total coliform group did not differ significantly from total aerobic bacteria in its persistence as an aerosol. This is seen in Table 14, which combines data from both phases of the Ft. Huachuca study. In this table the ratio standard plate count/Endo broth coliform bacteria is given for both nonchlorinated effluent and LEAP sample in the same runs, and the runs are grouped according to distance of the sampler from the sprayer. This ratio remains approximately the same as one moves from effluent to aerosol, within the range of sampling distances represented in the data. This does not eliminate the possibility that some groups of bacteria present in sewage might be more persistent in the aerosol state than the coliform group. Such organisms might well be of interest as possible indicators of microbial aerosols arising from wastewater sources.

Estimates of median particle diameter, derived from Andersen sampler data (see Table 9 for summarization) reveal no major differences between experimental and ambient (upwind) aerosols. There were also no differences in this respect between bacterial and viral aerosols. Comparison of bacterial aerosol data with the earlier phase of the study (downwind median = 4.7, upwind median = 4.4 μm) indicates no difference.

Mathematical prediction of net aerosol levels was performed on bacteria in four runs and on coliphage f2 in five runs. Predictions were made for each sampler location and employed separate calculations for each 1-minute interval as described in the earlier report.[6] The ratio predicted aerosol level/observed aerosol level proved to be quite variable (up to three orders of magnitude) from sampler to sampler within a single run (Tables 11, 12). This suggests that the model developed, which must make certain assumptions regarding the mechanism of aerosol generation by this sprayer, is quite imperfect in its apportionment of the aerosol plume to the various regions sampled. In accordance with this suggestion, understatement by the model of the downwind aerosol strength in one area would

TABLE 14. RATIO OF TOTAL AEROBIC BACTERIA TO ENDO BROTH COLIFORM BACTERIA IN NOZZLE GRAB SAMPLES AND LEAP AEROSOL SAMPLES. COMBINED DATA FROM PHASES I AND II

Distance (m)	Phase and Run	Std Plate Count/Total Coliform	
		Effluent	Aerosol
46	I-12	1100.0	2300.0
	I-13	49.0	40.0
	Geom. Mean	232.0	303.0
61-76	I-7	21.0	4.9
	II-12	12.0	59.0
	II-14	6.4	28.0
	Geom. Mean	11.7	20.1
91-107	I-9	46.0	43.0
	I-11	780.0	860.0
	I-14	240.0	62.0
	II-12	12.0	51.0
	II-14	6.4	27.0
	Geom. Mean	58.1	79.4
152-198	I-2	580.0	1.1
	I-10	3.5	310.0
	Geom. Mean	45.0	18.5
All Distances	Geom. Mean	47.0	55.2

be of necessity compensated for in some degree by overstatement as regards another downwind area. Therefore, organisms collected in the sampling array have also been presented (Table 13) as a composite sample (the sum of all downwind samples) and the result compared to a composite prediction (the sum of counts/m^3 prediction for all downwind samplers). The datum given in the table is the ratio total predicted recovery/total actual recovery. Here it is seen that excess of predicted over observed spans the range from 308- to 1661-fold for unchlorinated effluent.

This excess of predicted over observed can be regarded as a measure of overall efficiency, i.e., an overall relation between source strength and resultant aerosol. The major components of this relation are aerosolization efficiency of the sprayer, which the model assumes to be 100 percent, and biological die-off, which the model assumes to be zero. Physical decay is probably a lesser factor. Study of fluorescein aerosols permits observation of the overall efficiency in the absence of biological decay. However, in the present phase, the fluorescein runs

were not subjected to modeling. In the earlier phase of the study, the ratio predicted/observed for fluorescein runs was 310 (0.32 percent efficiency). Thus, biological die-off should be reflected in increases of the ratio predicted/observed above 310; cf. ratios of 308 to 1661 for unchlorinated effluent shown in Table 13.

No difference is apparent between bacterial and viral aerosols as reflected in these ratios. This is in contrast to laboratory data,[6] indicating a lower aerosol die-off rate for f2 than for E. coli or S. marcescens. The present study differs presumably because of the consistently low humidity levels encountered (Tables 3 and 5) and because of the difference in the spectrum of resistance by bacterial types involved.

In addition there appears to be no significant difference between the ratios for runs performed at dusk or in darkness and those for daytime runs. In contrast, actual aerosol levels, in relation to source strength, were higher under conditions of dusk or darkness (Table 7, runs 3, 5, 9, 10) than in daytime. This may be due largely to greater atmospheric stability or reduced wind velocity (Tables 4 and 5). Since the model takes these factors into account, higher predicted aerosol levels would result.

As regards the public health significance of these and similar observations, important gaps in our knowledge remain. These include: (1) the number of cells or virus particles of various pathogens in the aerosol state required to initiate infection in man and; (2) whether, and under what circumstances, foci of human infection actually arise in association with spray irrigation using wastewater.

CONCLUSIONS

In the absence of chlorination, aerobic bacteria were detected at greater than background levels at 150 m downwind.

The bacterial aerosol concentration was reduced to near background levels even at the immediate downwind stations when a chlorine residual was maintained in the wastewater.

Seeded coliphage f2 at about the same wastewater source density as standard bacterial plate count were readily detected in unchlorinated wastewater aerosols at 563 m downwind. At 46 m coliphage f2 reached levels of 1.9×10^4 pfu/m^3. Coliphage f2 aerosols from chlorinated water were detected at 137 m downwind. Coliphage f2 in the wastewater were reduced 18-fold by chlorination as opposed to 3300-fold for bacteria.

Coliphage aerosols were readily measured by impaction on agar surfaces in Andersen samplers, but the efficiency of recovery was only 20-25 percent as measured against liquid-impingement AGI samplers. There was, otherwise, good agreement between the different types of sampling equipment used.

The median particle diameter was ca 5.0 µm for both bacteria- and virus-bearing particles. Approximately 45-55 percent of such particles fell within 1-5 µm, the range of efficient pulmonary deposition. Thus pathogenic aerosol components, if present, could readily reach the human lung.

Aerosol concentrations of seeded fluorescent dye were somewhat greater in relation to source strength than microbiological aerosols. The difference was well within an order of magnitude, indicating a rather limited biological die-off.

Mathematical modeling of the aerosol plume yielded predictions of aerosol density that, when taken in conjunction with the aerosol-generating efficiency of the sprayer, as indicated by data from the earlier phase of the study, yields a factor of 1.0 to 5.5 for die-off and other aerosol decay factors.

None of the processes or safeguards considered, i.e., biological die-off in the aerosol state, distances up to 563 m, or chlorination, achieves more than a partial reduction in the numbers of microorganisms present in the aerosol.

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LIST OF ABBREVIATIONS

AGI	All-glass impinger
AGI-30	All-glass impinger, 30 ml
C	Degrees Celsius
cfp/m^3	Colony-forming particles per cubic meter
cfu	Colony-forming units
cfu/m^3	Colony-forming units per cubic meter
cfu/ml	Colony-forming units per milliliter
DPD	Diethylphenylenediamine oxalate
E. coli	*Escherichia coli*
Exp.	Experiment
Geom.	Geometric
Gram-pos.	Gram-positive
hrs	Hours
l/min	Liters per minute
LEAP	Lundgren Electrostatic Aerosol Precipitator Air Sampler (Environmental Research Corp.)
m	Meters
m^3	Cubic meters
mg/l	Milligrams per liter
min	Minutes
ml	Milliliters
mm	Millimeters
mps	Meters per second
µm	Micrometers
ng/ml	Nanograms per milliliter
ng/m^3	Nanograms per cubic meter
O-F	Oxidative-fermentative
pfu/m^3	Plaque-forming units per cubic meter
pfu/ml	Plaque-forming units per milliliter
pfp/m^3	Plaque-forming particles per cubic meter
RH	Relative humidity

RNA	Ribose nucleic acid
S. marcescens	*Serratia marcescens*
sec	Seconds
TYE	Tryptone-yeast extract